Exchange of Buffer System for MALDI-MS Analysis of Oligonucleotides without Desalting

Andreas Baumeister[1], Ido Bachelet[2], Ronen Weiss[2]
[1]Shimadzu Europa GmbH, Duisburg, Germany; [2]Augmanity Nano Ltd, Rehovot, Israel

Overview

- Magnesium is required to promote reactions of oligonucleotides.
- Magnesium chloride is causing ion suppression and desalting is needed.
- Successful MALDI-TOF analysis can be achieved without desalting by exchanging Magnesium chloride to Magnesium nitrate.

1. Introduction

During synthesis of oligonucleotides, buffers and salts are mandatory in many cases. Typically, the solvent contains buffers to stabilize the pH in a suitable range for the reaction. In addition, distinct cations such as Magnesium are required to support reactions. MALDI-MS tolerates certain amounts of those additives and allows for the analysis of mixtures so that a chromatography step might be avoided. However, simple desalting methods are frequently used to avoid adduct formation and ion suppression. Here, we present how a change in buffer composition can help to circumvent these desalting steps.

2. Materials and Methods

2.1 Sample preparation

Pure RNA with different lengths between 5 and 50 bases (Integrated DNA Technologies, Coralville, IA) were dissolved in pure water and in buffer solution to simulate usual situations in the lab and observe the influence of the buffer on the MALDI-MS analysis. The buffer contained TRIS, acetic acid, EDTA and $MgCl_2$. The effect of desalting was tested by the usage of ZipTip® (Merck Millipore). Samples were prepared with 3-HPA matrix.

2.2 MALDI-MS

MALDI-MS analysis was performed in positive and negative ion mode using a MALDI-8030 dual-polarity benchtop time-of-flight mass spectrometer (Shimadzu, Figure 1). Results were compared.

3. Results

3.1 Change from $MgCl_2$ to $Mg(NO_3)_2$

An RNA with 32 bases was analyzed in pure water (Figure 2), in $MgCl_2$ containing buffer (no signal, data not shown), in $MgCl_2$ containing buffer after desalting with ZipTip (Figure 3) and in $Mg(NO_3)_2$ containing buffer (Figure 4).

The comparison between the sample in pure water (Figure 2) and the buffered and desalted sample (Figure 3) shows that for higher masses the signal intensity decreases dramatically as recovery from the column was limited for bigger molecules.

Changing the Magnesium salt from Chloride to Nitrate shows a much better performance so that desalting is not needed anymore.

Figure 1. MALDI-8030 dual-polarity benchtop time of flight mass spectrometer

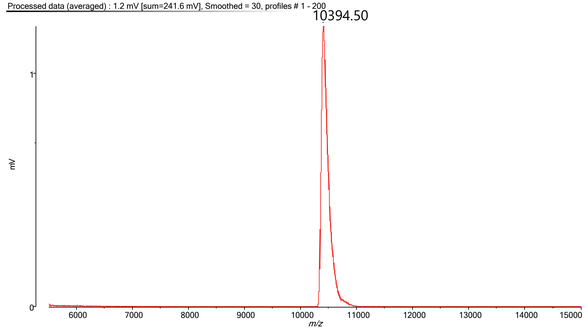

Figure 2. Direct analysis of RNA with 32 bases in pure water.

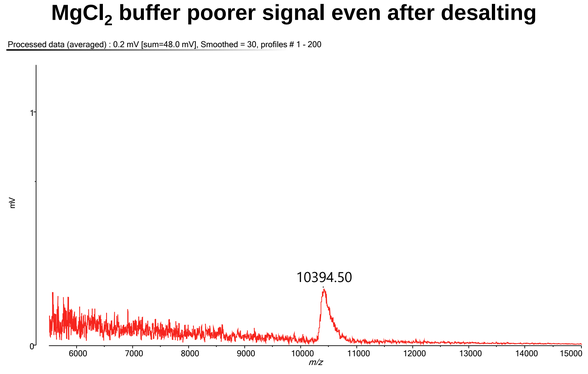

Figure 3. Analysis after desalting of RNA with 32 bases in $MgCl_2$ containing buffer.

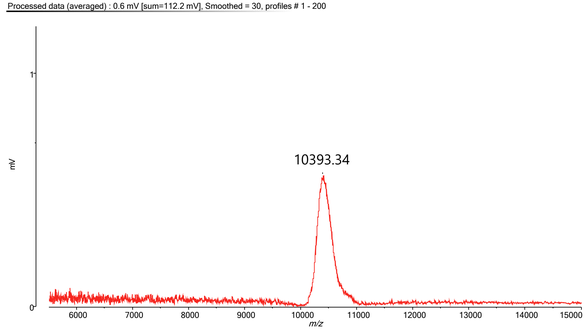

Figure 4. Direct analysis of RNA with 32 bases in $Mg(NO_3)_2$ containing buffer.

Of course, signal intensity and noise level was better without buffer (Figure 2). However, many applications require buffers to stabilize the pH value and certain cations like Mg^{2+} to promote reactions. This example shows that even with buffer a reasonable mass spectrum can be acquired. The choice of a MALDI compatible anion can help to ease sample preparation and save time and consumables.

3.2 Polarity switch to positive ion mode

Next to negative ion mode, also positive ion mode was tested for the same sample (Figure 5). The change to positive ion mode showed even higher intensity and signal-to-noise ratio than negative ion mode.

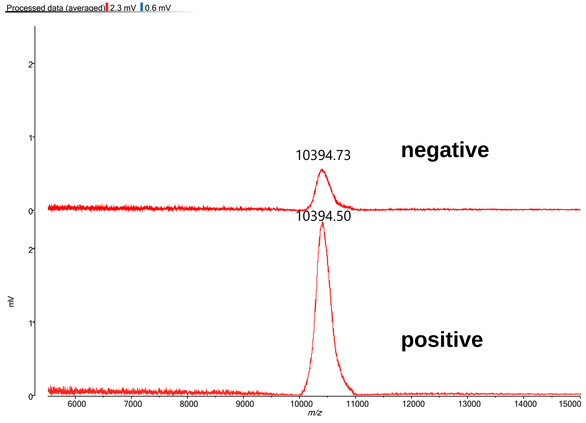

Figure 5. Direct analysis of RNA with 32 bases in $Mg(NO_3)_2$ containing buffer without desalting in positive and negative ion mode.

4. Conclusions

- Positive mode showed better intensity than negative mode.
- Desalting with ZipTip® shows lower recovery rate for longer RNA chains.
- Change from $MgCl_2$ to $Mg(NO_3)_2$ shows very good results even without desalting.

Andreas Baumeister is affiliated and funded by Shimadzu. Ido Bachelet and Ronen Weiss declare no competing financial interest.